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ORIGINAL ARTICLE

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Prevalence, antibiotic resistance, and MLST typing of *Helicobacter pylori* in Algiers, Algeria

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Abstract

Background: *Helicobacter pylori* infection is common in Algeria, but there are few data on the characterization of isolated strains. The aim of this study was to update data on the prevalence of *H. pylori* in patients submitted to endoscopy, antibiotic resistance, and phylogeography of *H. pylori* strains isolated in Algiers.

Materials and Methods: This is a prospective study carried out between November 2015 and August 2016. The culture of *H. pylori* was performed on antral and fundic gastric biopsies of adult patients from 3 hospitals. A real-time PCR using the fluorescence resonance energy transfer (FRET) principle for the detection of *H. pylori* followed by a melting curve analysis for the detection of mutations associated with resistance to clarithromycin was applied. Differentiation between antral and fundic isolates of the same patient was also determined by RAPD, and an MLST typing was performed for characterization of the phylogeographic group of *H. pylori*.

Results: By real-time PCR, the prevalence of *H. pylori* infection among the 147 patients included was 57%. Culture was positive in only 29% of the cases. Twenty-seven percent of patients had received *H. pylori* eradication treatment. The primary and secondary resistance rates to clarithromycin were 23% and 36%, respectively, and to metronidazole, 45% and 71%, respectively. Only one isolate was resistant to levofloxacin, and no resistance to amoxicillin, tetracycline, and rifampicin was detected. A double population was present in 14 patients. The MLST analysis classified the 42 *H. pylori* strains from 38 patients in 2 haplotypes: hpEurope (33) and hpNEAfrica (9).

Conclusion: The prevalence of *H. pylori* remains high in Algeria but appears to be decreasing in recent years. High resistance to clarithromycin requires increased monitoring of the evolution of antibiotic resistance and adaptation of eradication therapy.

KEYWORDS
culture, macrolide, multiple infection, random amplified polymorphic DNA, real-time PCR

1 | INTRODUCTION

Since the discovery of *Helicobacter pylori* in 1982,[1] its role in gastroduodenal pathologies such as gastritis, peptic ulcer, MALT lymphoma and gastric cancer has been clearly established.[2] Although *H. pylori* infection is the most frequent chronic bacterial infection in the world with approximately 50% of the world population infected, prevalence varies considerably from one country to another depending on the

socioeconomic level. It is very high in developing countries where it can reach 90%, whereas it may be <20% in industrialized countries.[3]

The choice of the appropriate eradication therapy is based mainly on the resistance rate of the bacterium to antibiotics in the region, especially to clarithromycin, an antibiotic which has an increasing resistance rate in many countries[2] leading to eradication failures.

Algeria is one of the countries with a high prevalence of *H. pylori* infection,[4] but there are very few recent data on trends of prevalence and on characterization of the strains. The aim of this study was to update the data on the prevalence and resistance of *H. pylori* to antibiotics and for the first time to perform a phylogeographic characterization of the strains isolated in Algiers by multilocus sequence typing (MLST).

2 | MATERIALS AND METHODS

2.1 | Patients/biopsies

This multicentric study included adult patients who were referred for gastroduodenal endoscopy at 3 hospitals in Algiers (Bab El Oued and Béni Messous Gastroenterology departments and Bologhine Internal Medicine department) over the period November 2015 to August 2016. One antral and one body biopsy were transported in a brain-heart infusion (BHI) broth (Institut Pasteur d'Algérie, Algiers, Algeria) at +4°C accompanied by a patient information sheet to the clinical biology laboratory of the Bologhine Hospital on the same day.

2.2 | Culture and antibiogram

The antral and the body biopsies were ground separately in 1 ml of BHI. A portion of the suspension was frozen at −80°C to carry out molecular biology tests at the French National Reference Center for Campylobacter and Helicobacter (Bordeaux, France). The other part was cultured on Colombia agar medium supplemented with 10% human blood and a selective supplement (*Helicobacter pylori* selective supplement, Oxoid, England). The cultures were incubated at 37°C in a microaerobic atmosphere (CampyGen, Oxoid, Basingstoke, UK) for 3 to 10 days. The identification of suspicious colonies was based on the typical morphology on Gram stain preparations and the production of oxidase, catalase, and urease. The identified strains were stored at −80°C in BHI supplemented with 20% glycerol. The antimicrobial susceptibility testing (AST) was performed by the agar diffusion method on Mueller-Hinton medium supplemented with 10% human blood with a bacterial suspension equivalent to a McFarland 3 opacity standard. Amoxicillin, tetracycline, rifampicin, levofloxacin, and clarithromycin were tested using ATB disks (bioMerieux, Marcy-l'Etoile, France); Etest was used for metronidazole and to confirm resistance to clarithromycin and levofloxacin. Critical concentrations were interpreted according to EUCAST guidelines, clarithromycin: resistant <17 mm, susceptible > 22 mm; tetracycline: resistant < 17 mm, susceptible > 19 mm; rifampicin: resistant < 14 mm, susceptible > 19 mm; levofloxacin: resistant < 17 mm, susceptible > 20 mm; amoxicillin: resistant < 17 mm, susceptible > 20 mm.

2.3 | DNA extraction

The fragments of each biopsy or the suspensions of each strain were digested in 20 µl of proteinase K and 180 ml of lysis buffer. DNA extraction from biopsy specimens was performed with MagnaPur 96 (Roche Diagnostics, Meylan, France) and from strains using the DNA extraction kit QIAampDNAmini-kit (Qiagen, France) according to the manufacturer's instructions.

2.4 | Real-time PCR detection of *H. pylori* and its resistance to clarithromycin

The detection of *H. pylori* from gastric biopsies and confirmation of the identification of strains obtained by culture, as well as the determination of point mutations in the 23SrRNA gene associated with clarithromycin resistance, were performed by real-time PCR as previously described.[5] Real-time PCR detection of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed on negative biopsy specimens to control for DNA extraction.

2.5 | Differentiation of strains using random amplified polymorphic DNA (RAPD) genotyping

Genomic differences between the antral and the body strains isolated from the same patient were investigated by RAPD PCRs with 2 primers 1254 and 1290.[6] The PCR products were analyzed on a 1% agarose gel.

2.6 | Detection of quinolone resistance

A PCR of the quinolone resistance determining region (QRDR) of the *H. pylori gyr*A gene followed by sequencing of the PCR product was performed for the detection of quinolone resistance as previously described.[7]

2.7 | Genotyping by MLST

Phylogeographic typing was performed by MLST. PCR amplification and sequencing of 7 *H. pylori* housekeeping genes (*atp*A, *efp*, *trp*C, *ppa*, *mut*Y, *yph*C and *urel*) were performed as previously described.[8] Strain population assignment was performed using the "no admixture model" of STRUCTURE,[9] as previously described.[10] Using the same conditions, the "admixture model" was also applied to verify the existence of admixture of distinct populations. Phylogenetic tree reconstitution was made using the neighbor-joining algorithm implemented in MEGA 6.0 software.

3 | RESULTS

3.1 | Characteristics of the patients

A total of 147 patients were included in this study. Endoscopy was performed for 100 patients (68%) for epigastralgy, 29 patients (20%)

for eradication control, 11 patients (7%) for gastroesophageal reflux, 4 (3%) for Biermer anemia, and 3 patients (2%) for vomiting. One hundred and seven patients (73%) had never received *H. pylori* eradication therapy; 43% of them were male with a mean age of 49 years (extremes 17-90 years). Forty patients (27%) had received *H. pylori* eradication therapy; 50% were male with a mean age of 49 years (extremes 18-72 years). Thirty-two patients (80%) had received a first-line therapy with proton-pump inhibitor (PPI), amoxicillin and metronidazole, 6 (15%) with PPI, amoxicillin, and clarithromycin, and for 2 patients (5%), the treatment prescribed was not determined.

The gastric lesions revealed by endoscopy are presented in Table 1.

3.2 | *H. pylori* prevalence and antibiotic resistance

Among the 107 patients who did not receive an *H. pylori* treatment, 60 (56%) were positive by real-time PCR and 27 (25%) by culture. Prevalence varied between 47% and 65% by age group. Mutations A2142/43G associated with clarithromycin resistance were detected in 14 patients (23%). A double population, both susceptible and resistant to clarithromycin, was detected in 6 patients (10%). The AST for the strains which grew revealed that 9 strains (33%) were resistant to clarithromycin and 11 strains (41%) were resistant to metronidazole with a MIC > 256 μg/ml. No resistance to amoxicillin, tetracycline, levofloxacin, and rifampicin was detected among the 27 strains tested (Table 2). A double population of *H. pylori* strains in the antrum and body was revealed by RAPD in 3 of the 8 patients tested.

Among the 40 patients who had previously received an *H. pylori* treatment, 22 (55%) were positive by PCR and 16 (40%) by culture. Prevalence varied between 36% and 67% by age group. Using real-time PCR, secondary resistance to clarithromycin was detected in 8 patients (36%) (mutation A2142/43G). Two patients (9%) were infected by a double population, both susceptible and resistant to clarithromycin. Using AST, 7 strains (44%) were resistant to clarithromycin, 12 (75%) to metronidazole with a MCI > 256 μg/ml, and only one strain was found to be resistant to levofloxacin (MIC > 256 μg/ml). Sequencing of the QRDR region revealed an *Asn87Thr* mutation. No resistance to amoxicillin, tetracycline, and rifampicin was detected among the 16 strains tested (Table 2). RAPD revealed antral and body co-infection by a double population of *H. pylori* in 1 of the 10 patients tested.

No discordance between AST results and real-time PCR detection of clarithromycin resistance was found.

3.3 | Phylogeographic typing

The "no admixture model" of STRUCTURE of the 42 strains isolated from 38 patients of North African origin, based on the sequences of the 7 housekeeping genes included in the MLST scheme for *H. pylori*, grouped the Algerian strains into 2 haplotypes: 33 hpEurope strains and 9 hpNEAfrica strains. The "admixture model "revealed that 13 hpEurope strains were a mosaic between hpEurope, hpNEAfrica, and hpAfrica1 (Table 3). The phylogenetic tree of the 42 strains is represented in Figure 1. It should be noted that the strains ALG6 and ALG8 isolated in different patients without any link between them are surprisingly very close in the phylogenetic tree.

TABLE 1 Distribution of patients according to endoscopy results

Endoscopy diagnosis	Patients not previously treated N (%)	Patients previously treated N (%)
Normal	5 (5)	1 (2.5)
Gastritis only	91 (85)	28 (70)
Duodenal ulcer	8 (7)	7 (17.5)
Gastric ulcer	3 (3)	1 (2.5)
MALT lymphoma	0 (0)	3 (7.5)
Total	107 (100)	40 (100)

4 | DISCUSSION

The prevalence rate of *H. pylori* infection in this study (56%) remains high as is the case in the majority of developing countries. However, there has been a significant decrease in recent years. Serologic studies in the 1980s reported a prevalence of *H. pylori* infection in the Algerian population >80%,[4] while more recent studies report lower rates.[11] This reduction can be explained by the improvement in hygiene conditions which minimizes transmission, better therapeutic management of the patients, and the unintentional eradication of the bacteria by

TABLE 2 *Helicobacter pylori* primary and secondary antibiotic resistance

	Primary resistance		Secondary resistance	
Antibiotic	Tested N	Resistant N (%)	Tested N	Resistant N (%)
Clarithromycin[a]	60	14 (23)	22	8 (36)
Clarithromycin[b]	27	9 (33)	16	7 (44)
Metronidazole[b]	27	11 (41)	16	12 (75)
Levofloxacin[b]	27	0 (0)	16	1 (6)
Amoxicillin[b]	27	0 (0)	16	0 (0)
Tetracycline[b]	27	0 (0)	16	0 (0)
Rifampicin[b]	27	0 (0)	16	0 (0)

[a]PCR.
[b]Antibiogram.

TABLE 3 Population structure of 42 Algerian *Helicobacter pylori* strains

H. pylori strains	Patients endoscopy diagnosis	Age	Sex	Patient previously treated	Population structure (no admixture model)
AlG 1[a]	MALT lymphoma	72	Male	Yes	hpNEAfrica
ALG 2[a]					hpNEAfrica
ALG 3	Gastritis	70	Male	Yes	hpEurope
ALG 4	MALT lymphoma	21	Male	Yes	hpEurope
ALG5	Gastritis	35	Female	Yes	hpEurope[e]
ALG 6[b]	Gastritis	50	Female	No	hpNEAfrica
ALG 7[b]					hpEurope[e]
ALG 8	Duodenal ulcer	45	Female	Yes	hpNEAfrica
ALG 9	MALT lymphoma	45	Female	Yes	hpEurope
ALG 10	Gastritis	35	Female	Yes	hpEurope
ALG 11	Gastritis	30	Male	Yes	hpEurope
ALG 12	Duodenal ulcer	40	Male	No	hpEurope
ALG 13	Duodenal ulcer	59	Male	No	hpNEAfrica
ALG 14	Gastritis	30	Male	Yes	hpEurope[e]
ALG 15	Gastritis	37	Male	No	hpEurope
ALG 16	Gastritis	50	Female	No	hpEurope[e]
ALG 17	Gastritis	53	Female	Yes	hpEurope[e]
ALG 18[c]	Duodenal ulcer	65	Female	No	hpEurope[e]
ALG 19[c]					hpEurope
ALG 20	Gastritis	37	Male	No	hpEurope
ALG 21	Duodenal ulcer	49	Female	No	hpEurope[e]
ALG 22	Gastritis	46	Female	No	hpNEAfrica
ALG 23[d]	Gastritis	35	Female	No	hpEurope[e]
ALG 24[d]					hpEurope
ALG 25	Duodenal ulcer	44	Male	No	hpEurope[e]
ALG 26	Gastritis	45	Female	No	hpNEAfrica
ALG 27	Gastritis	54	Female	No	hpEurope
ALG 28	Gastritis	19	Female	No	hpNEAfrica
ALG 29	Gastritis	35	Male	No	hpEurope
ALG 30	Gastritis	42	Female	Yes	hpEurope
ALG 31	Gastritis	48	Female	No	hpEurope[e]
ALG 32	Gastritis	59	Female	Yes	hpEurope
ALG 33	Gastritis	56	Male	Yes	hpNEAfrica
ALG 34	Duodenal ulcer	58	Male	Yes	hpEurope[e]
ALG 35	Gastritis	58	Male	Yes	hpEurope
ALG 36	Gastritis	57	Female	No	hpEurope[e]
ALG 37	Duodenal ulcer	48	Female	No	hpEurope
ALG 38	Gastritis	26	Female	No	hpEurope
ALG 39	Gastritis	72	Female	No	hpEurope
ALG 40	Gastritis	66	Female	No	hpEurope
ALG 41	Gastritis	63	Male	No	hpEurope
ALG 42	Gastritis	47	Female	Yes	hpEurope[e]

(a-d) Each couple of strain was isolated from a same patient; strains were different with RAPD.
[e]Mosaic: hpEurope, hpNEAfrica, hpAfrica1 (admixture model).

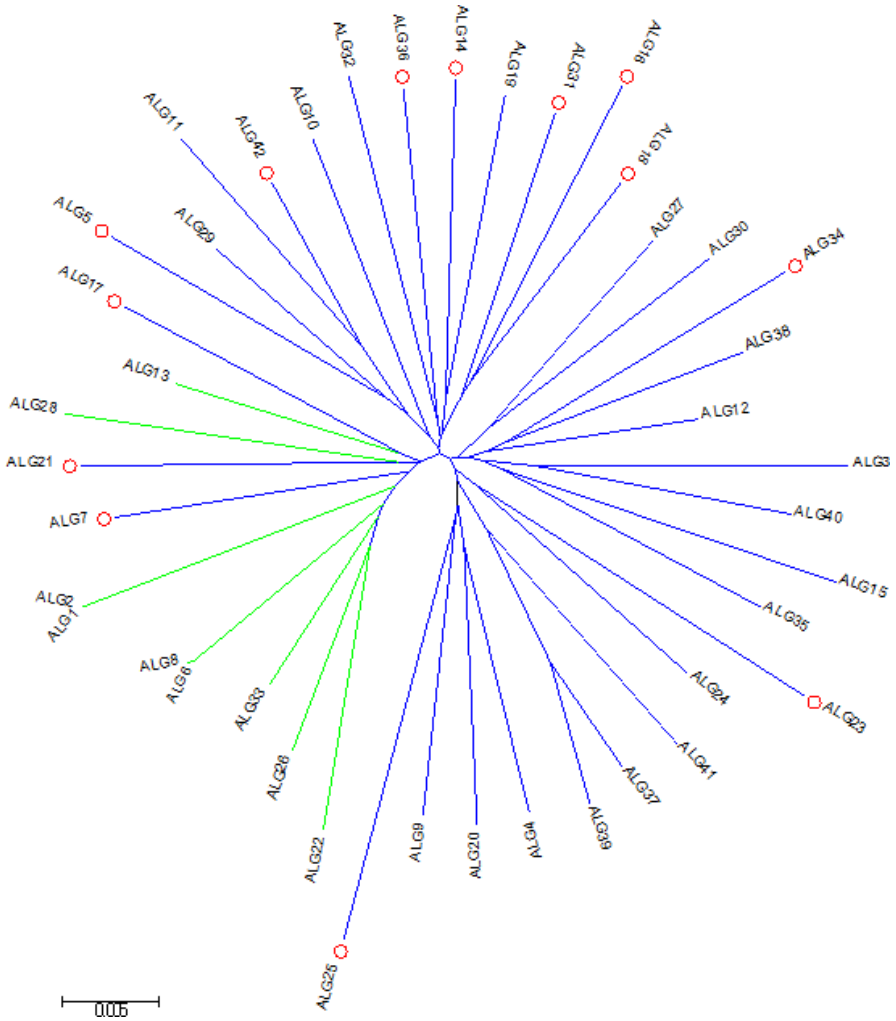

FIGURE 1 MLST analysis of 42 Algerian strains of *Helicobacter pylori*. Phylogenetic tree constructed using neighbor-joining tree with MEGA v6. — hpEurope, — hpNEAfrica, O mosaic: hpEurope, hpNEAfrica, hpAfrica1. (ALG1 and ALG2), (ALG6 and ALG7), (ALG18 and AG19), and (ALG23 and ALG24): each couple of strain was isolated from a same patient; strains were different with RAPD

the increased use of antibiotics for the treatment of other infections. Similar decreases in the prevalence of *H. pylori* infection have been observed in several developing countries: In Ghana, it decreased from 69.7% in 1999 to 45.2% in 2012.[12] A Brazilian study reported a 5.2% decrease in prevalence between 2004 and 2014.[13] In Iran, too, a large decline in prevalence has been described in several studies.[14,15] The prevalence in our study concerns only symptomatic patients and does not represent those of the general population. A Tunisian study showed a significantly higher prevalence in a symptomatic group of patients compared to a group of asymptomatic blood donors, 99% vs 64%.[16] In the present investigation, the prevalence is similar in untreated patients and in patients previously treated with *H. pylori* eradication, which means that almost half of the patients treated are still infected with the bacteria. This raises the question of the efficacy of the prescribed eradication therapy and patient compliance as well as the possibility of re-infection. Indeed, several studies reported a high risk of re-infection after eradication in high-prevalence countries. The rate of re-infection is relatively low 1 year after eradication[17] but increases significantly after 3 years in high-prevalence countries.[18,19] The absence of precise data for all patients concerning the given eradication therapy and the time lapse between treatments and biopsy sampling in our study does not allow us to favor one hypothesis over the other. The distribution of prevalence by age groups in this study shows that it is acquired very early and persists for a long time before beginning to decline in the sixties.

The level of *H. pylori* detection by culture is considerably lower than by PCR (29% vs 56%, respectively). *H. pylori* is a very fragile bacterium and extremely dependent on transport conditions. The lack of molecular biology techniques at the Bologhine Hospital laboratory makes culture the only alternative for diagnosis and AST, particularly for patients in treatment failure. However, another advantage of culture is the conservation of strains for subsequent molecular typing studies.

The choice of an *H. pylori* eradication therapy is based primarily on the rate of clarithromycin resistance in the region. Above 15% of resistance, a triple therapy based on clarithromycin is not recommended.[2] In Algeria, in the absence of updated data on *H. pylori* antibiotic resistance, a triple therapy based on clarithromycin or metronidazole is widely used. In this study, we report a 23% primary resistance to clarithromycin. Another Algerian study recently reported an even higher rate of 36%.[11] Many other countries have high resistance rates to clarithromycin: 29% in Morocco,[20] 22.2% in France,[21] 17.9% in Spain[22]; in others, the rate remains relatively low and stable and <5% in Sweden.[23] A European study highlighted the relationship

between macrolide use in the community and the high prevalence of *H. pylori* resistance to clarithromycin.[24] Due to a lack of data on macrolide consumption in Algeria, this can only be a supposition to explain the high rate. All of the resistances corresponded to one of the mutations A2142/43G; unfortunately, the technique used is unable to differentiate them. According to these data, triple therapy using clarithromycin is no longer adequate in Algeria without first carrying out AST. The primary resistance to metronidazole in this study is also very high (45%). However, unlike clarithromycin, the impact of metronidazole resistance can be overcome by increasing the duration of treatment. On the other hand, levofloxacin resistance is extremely low, a single strain with an *Asn87Thr* mutation was found, contrary to the increase observed in several regions: 15.4% in France,[21] 22.1% in Italy, and 34.5% in China.[23] Our results suggest that levofloxacin can be introduced into the *H. pylori* eradication treatment in Algeria. Obtaining the antibiotic profile of an *H. pylori* strain is difficult in Algeria because few laboratories carry out the bacterial culture or the PCR for detecting mutations conferring resistance to antibiotics. In view of the previous Algerian studies as well as our results, and according to the latest recommendations of Maastricht V,[2] it is mandatory to adapt the *H. pylori* eradication treatment in Algeria. Quadruple concomitant therapy (amoxicillin 1000 mg, clarithromycin 500 mg, metronidazole 500 mg, and omeprazole 40 mg twice a day for 14 days) would be the first-line eradication treatment given that bismuth is not available.

The MLST phylogenetic analysis of the 42 strains from 38 patients revealed that the most predominant haplotype was hpEurope with a large share of mosaic strains, which is in agreement with the North African location of Algeria. This distribution is the result of human migrations since the Paleolithic period illustrated in studies conducted by Falush et al[25] and Moodley et al,[26] as well as the more recent mixing of North African and South European populations with the common history of these 2 regions.

In this study, we demonstrated the double colonization of *H. pylori* in the same patient in different ways. Real-time PCR detected clarithromycin susceptible and resistant variants of the same strain in 10% of patients, which may have an impact on eradication. RAPD revealed that the *H. pylori* strain colonizing the antrum was different from the one colonizing the body in 4 patients. The strains of 3 of these patients had the same antibiotic profile so the difference could not be detected by AST. The results were confirmed by MLST for 3 patients (Figure 1): One patient was co-infected with one strain of hpNEAfrica haplotype (ALG6) and one strain of hpEurope haplotype (ALG7), and 2 patients were co-infected with 2 different strains of hpEurope haplotype (ALG18 and ALG19, ALG23 and ALG24). Interestingly, strains ALG23 and ALG24, isolated in the same patient, are quite near in the phylogenetic tree. Concerning the 4th patient, the antral and the body strains (ALG1 and ALG2) were identical by MLST (Figure 1). The genetic difference between them probably involved other parts of the bacterial genome than the 7 housekeeping genes sequenced by MLST. The application of several techniques is sometimes necessary to demonstrate infections by multiple strains of *H. pylori*. Cases of multiple infections are very common and are more prevalent in high-prevalence countries.[27,28] In this respect, a French–Tunisian study reported a 48% multiple infection rate in Tunisia, a country with a high prevalence, compared to 5% in France, a country with a low prevalence.[27] These multiple infections facilitate gene transfer between strains and contribute to the genetic diversity of the bacteria.[29-31]

5 | CONCLUSION

According to the results of this study, the prevalence of *H. pylori* infection remains high in Algeria but appears to have decreased in recent years. High clarithromycin resistance requires increased monitoring of the evolution of antibiotic resistance and adaptation of eradication therapy according to current data. Obviously, these results should be confirmed and supplemented by other studies with larger numbers of patients and covering several regions of the country.

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The authors have no competing interests.

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